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Pathological and Virological Studies on an Outbreak of Lumpy Skin Disease among Cattle in Kalubia Governorate-Egypt

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ABSTRACT

This work was proposed to study pathologic characteristics of lumpy skin disease (LSD) among naturally infected cattle and to demonstrate LSDV antigen within different tissues including the skin nodules, regional lymph nodes, lung and liver using PCR and immunohistochemistry. Among the infected cattle, one dairy cattle and 5 calves were dead (mortality rate is 6.97%). The major gross alterations in all investigated cases included numerous 1-5 cm well circumscribed, round cutaneous nodules covered the whole body in most cases with severe enlargement of superficial lymph nodes. The main microscopical changes were severe ballooning degeneration of the epidermis, dermatitis, with severe vasculitis affecting the dermal blood vessels. Multiple intracytoplasmic eosinophilic inclusions were present in degenerated epidermal cells. Several pathological changes were also detected in the liver, lung, kidney, intestine and lymph nodes. Strong positive PCR signals were appeared in skin nodules, lung and lymph node. Additionally, positive immunoperoxidase reaction was demonstrated in the skin, lung, kidney and lymph nodes. Furthermore, LSDV was isolated on chorio-allantoic membrane (CAM) of spf embryonated chicken eggs from skin nodules and regional lymph nodes collected from dead animals. Virion particles were observed on CAM by electron microscope. Finally, it could be concluded that skin lesions is a constant lesion in both young and adult animals together with systemic infection in severely infected animals.

Introduction

Lumpy skin disease (LSD, Pseudo-urticaria, Neethling virus disease, exanthema nodularis bovis and knopvelsiekte) is an infectious viral disease of cattle. It is caused by Lumpy skin disease virus (LSDV) belonging to the family poxviridae, within the subfamily Chordopoxvirinae and genus capripoxvirus that includes sheep and goat pox viruses (Tuppurainen and Oura 2012). The LSD virus is one of the hugest viruses known (170–260 nm by 300–450 nm) (Matthews, 1982).

LSDV exists in skin nodules, crusts of skin lesions, blood, saliva, nasal discharge, semen and milk (Babiuk *et al.*, 2008). LSD is observed also in Asia and appeared in non-African countries such as Madagascar, the Middle East and Israel (Davis, 1991; Yeruham *et al.*, 1995; Brenner *et al.*, 2006; Body *et al.*, 2012). Severe outbreaks were recorded in various provinces in Egypt (Ali *et al.*, 1990; House *et al.*, 1990; Salib and Osman, 2011, El-Nahas *et al.*, 2011). It usually occurs at regular intervals in endemic areas or it may cause epidemics, which spread rapidly throughout a region or country (Davies, 1991). LSDV is thought to be transmitted primarily by biting insects as it was detected in mosquitoes during some outbreaks (Tuppurainen

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et al., 2011). The incubation period in natural cases is thought to be between two to five weeks (Tuppurainen *et al.*, 2005). LSD takes different forms such as an acute, subacute or chronic disease of cattle. The morbidity rates in natural outbreaks vary from 3–85%. The disease has significant economic implications not only in endemic areas such as sub-Saharan Africa and Egypt, but also internationally (Agag *et al.*, 1992).

There are a variety of molecular methods were described to study the localization of LSDV capsid antigen within the different tissues using immunohistochemistry (IHC) and polymerase chain reaction (PCR). Routine histopathology and immunohistological staining provide a relatively inexpensive tool to diagnose the disease. Additionally, antigen trapping ELISAs has been developed more recently for detecting LSD antibodies (Heine *et al.*, 1999).

However, the pathology of LSD was illustrated in few literatures (Ali *et al.*, 1990), and pathological alterations in different internal organs are inadequately described till now. Therefore, this work was designed to investigate fully the pathologic characteristics of LSD in different organs (skin, regional lymph nodes, tongue, lung, liver, kidney and intestine) among naturally infected cattle and to detect the antigen of LSDV within different organs using PCR and IHC. Additionally, viral isolation as well as viral identification using different methods was also performed.

Materials and methods

Samples collections

The samples were collected from 86 native and foreign breeds of cattle in Kaluobia governorate farms and some of them were admitted to the clinic in Faculty of Veterinary Medicine Benha University. Firstly, these animals were examined clinically for the presence of LSD lesions during the period of June to December 2014. Their age ranged from 1-6 years of both sexes. The animal examination was concentrated on physical conditions, temperature, superficial lymph node and skin lesions according to (Rodostits *et al.*, 1995). Finally, the specimens were taken from different organs for histopathological examination, PCR and virus isolation.

Polymerase chain reaction (PCR)

Tissue samples were taken from the skin nodules, lymph nodes, lung and liver and stored at -20°C for analysis by PCR. DNA was extracted from these samples with the QIAmp® extraction kit (Qiagen, Vienna, Austria). According to manufacturer's instructions, 25 mg of each organ were used for extraction. PCR reaction was applied in a total volume of 50 µl containing: 1X PCR buffer (20 mM Tris HCl pH 8.4 and 50 mM KCl); 1.5 mM MgCl₂; 0.2 mM deoxynucleosides triphosphates mixture (dATP, dCTP, dGTP and dTTP); 20 pmol of each primer; 2.5 units (U) *Thermus aquaticus* Taq polymerase (Promega) 0.1 µg of extracted viral DNA and nuclease-free sterile double distilled water up to 50 µl. The PCR was performed using with commercially available primers for LSDV. The primers were developed from the gene for viral attachment. The forward and reverse primers had the sequences 5'-TTTCCTGATTTTCTTACTAT-3' and 5'-AAATTATATACGTAAATAAC-3', respectively. It was manufactured in the laboratories of the Midland Certified Reagent company Inc. of Midland, Texas. Then, the resulting mixture was subjected to precise thermal profile in a TGradient thermocycler (Biometra, Goettingen, Germany) as follows: an initial denaturation cycle of 94 °C for 2 min; followed by 40 cycles at 94°C for 50 seconds, 50 seconds at 50°C and 1 minute at 72°C for; followed by one final extension cycle of 72° C for 10 minutes, rendering an amplicon of 192 bp (Ireland and Binopal 1998). Amplified products (10 µl) were analysed using a 100 bp DNA ladder as a molecular marker on 1.5% agarose gels. Amplicons which were stained with ethidium bromide, were visualized using an UV transilluminator at a wavelength of 590 nm and positive reactions were confirmed according to size.

Pathological examination

Tissue samples were taken from the skin, lymph nodes, tongue, lung, liver, kidney and intestine. Samples from these organs were also fixed in 10% neutral buffered formalin solution. The fixed specimens were trimmed, washed and dehydrated in ascending grades of alcohol, cleared in xylene, and subsequently embedded in paraffin wax then sectioned (4-6 micron) and stained with hematoxyline and eosin according to Bancroft *et al.* (1996) for

detection of any microscopical lesions in the examined tissues. Specimens from skin nodules were taken from both live and dead animals, while necropsy from different internal organs (lymph nodes, tongue, lung, liver, kidney and intestine) was performed only from dead cases (six cases).

Immunohistochemistry (IHC)

Immunohistochemistry (IHC) was applied for definitive demonstration of the virus antigen in different organs including 20-selected specimen of skin nodules and six samples of lymph node, lung, kidney and liver. Following the protocol of Awadin *et al.* (2011) with some modifications, 3 µm-thick tissue sections Paraffin sections were mounted on positively charged glass slides (Superfrost plus; Menzel-Glaser, Braunschweig, Germany). Post deparaffinization and rehydration, retrieval of antigen was performed by heating the slides in citrate buffer (pH 6.0) in a microwave oven. Endogenous peroxidase activity was blocked with 1.5% H₂O₂ in methanol for 30 minutes. The sections were then incubated with 1:10 dilution of a normal goat serum (Sigma, G9023) mixed with 2% bovine serum albumin for 45 minutes at room temperature in a humidified chamber, followed by an overnight incubation with the primary LSDV antiserum (1:1000). To visualize the primary antibody, the sections were extensively washed in PBS and then the sections were incubated with a 1:500 dilution of biotinylated anti-rabbit IgG secondary antibody (Vector Laboratories, Burlingame, USA) for 30 minutes incubation at room temperature. followed by Vectastain ABC Kit (Vector Laboratories, Burlingame, USA) for 30 minutes. The reaction was visualized with a DAB Substrate Kit for peroxidase (Vector Laboratories, Burlingame, USA). Then counterstaining was with hematoxylin stain. To avoid cross contamination during necropsy examination, autoclaved scissors and disposable forceps were used for sampling.

Virological examination

Tissue Specimens

Skin nodules, liver, lung, kidney and lymph nodes were aseptically collected from infected animal. Each sample was prepared for virological examination (Carn and Kitching 1995) and preserved at -70°C till used.

Viral Isolation

For virus isolation, spf Eight-day-old embryonated chicken eggs (ECE) were inoculated with the prepared samples via the chorio-allantoic membrane (CAM) route according to House *et al.* (1990).

Serological identification of virus isolates

Agar gel precipitation test (AGPT and indirect fluorescent antibody technique (IFAT) were used for viral identification. For this, LSDV Neethling reference strain and reference antiserum to LSDV were kindly obtained from the Department of virology, Animal health Research Institute, Dokki, Giza. Anti-bovine IgG conjugated with fluorescent Isothiocyanate developed in rabbits (Sigma Aldrich) was used in IFAT. IFAT was carried out on infected CAM according to (Davies *et al.* 1991). The soluble antigen was prepared and identified from infected CAM using AGPT according to (Davies, 1981).

Electron microscope

Inoculated CAM was prepared and examined under the transmission electron microscope according to (Kitching and Smale 1986), at transmission electron microscope unit, national research center, Egypt.

Results

Clinical findings

The infected cattle were firstly suffered from fever that persisted for 7-12 days. Inappetance, salivation, cachexia and emaciation of infected animals were prominent. Lacrimal and nasal discharges in combination with respiratory manifestations were also noticed. Six animals were dead including one dairy cow and five calves; accordingly, the mortality rate is 6.97%.

PCR Assay

The PCR amplified viral DNA fragment of 192bp corresponding to the expected amplification product size of LSDV that was demonstrated in most examined organs including skin nodules, lung and

lymph nodes with different degrees, while there were no amplicon detected in liver as shown in Fig.1. The highest rate of the expected amplification product (100%) was found in the skin nodules. Furthermore, the same size (192 bp) of attachment protein gene fragment, were also found in 97% and 75% of samples processed from the lymph nodes and lung, respectively.

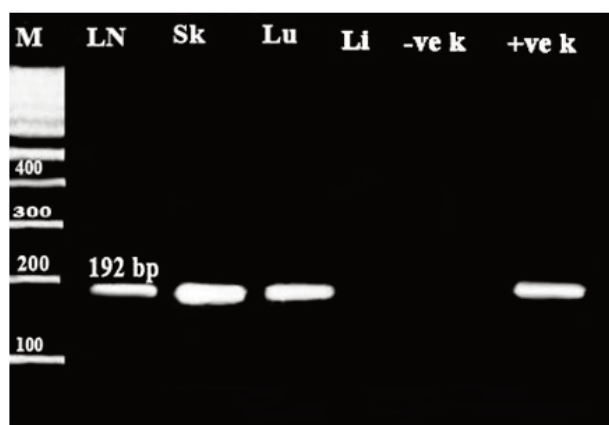


Fig. 1. Electrophoretic pattern of PCR amplification where Lane M: nucleic acid marker (100bp), Lane LN: lymph node sample, Lane Sk: original skin nodule sample, Lane Lu: lung tissue, Li: liver sample, Lane -ve k: negative control sample, Lane +ve k: Positive control (Reference virus).

Pathological findings

Gross lesions

Intradermal rapid eruption of multiple circumscribed, firm and of variable sized skin nodules. These nodules scattered all over the animal body surface (Fig. 2A). The distribution of the nodules varied among the infected animals as some animals mildly infected and have nodules only on the neck and thorax, while others are severely infected and have nodules scattered all over the body. In advanced cases, the affected cattle showed high incidence of scab formation after rupturing of nodules (Fig. 2B). As the disease progresses, the nodules became necrotic, and eventually a deep scab was detected. Some of the examined cases showing few number of nodules scattered on the head and neck, after a time these nodules covered whole the body. The skin lesions in dead cases characterized by sudden appearance of large number of nodules all over the body including eyes, ears, muzzle, thorax, abdomen, legs and scrotum. Some of these nodules may be ruptured leaving ulcers on the neck, head, and thorax. Generalized lymphadenitis was detected. Superficial lymph nodes especially

prescapular and prefemoral were enlarged.

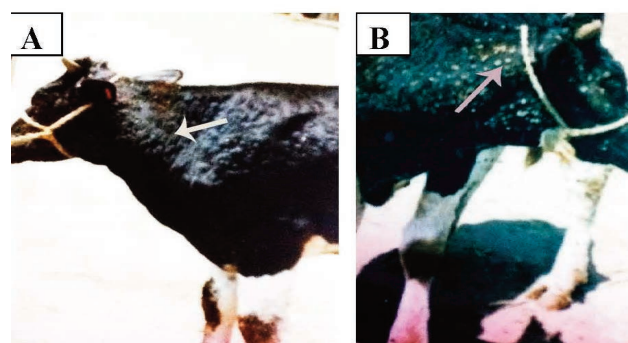


Fig. 2. Calf suffering from LSD, A) Multifocal nodules scattered all over the body (arrow), B) Dairy cow has flat alopecic cutaneous lesions that are covered with a scab (arrow).

Histopathological findings

The microscopical examination of the skin of cattle suffering from lumpy skin disease revealed hydropic degeneration of the epidermis particularly stratum spinosum and basal cell layer. Moreover, in some examined dead cases revealed ballooning degeneration of stratum spinosum with microvesicles formation. The vesicles were filled with serofibrinous exudates. All examined skin biopsy from infected animals as well as skin samples from dead animals showed presence of numerous intracytoplasmic eosinophilic inclusion bodies (Fig. 3A). However, these lesions were demonstrated in all examined skin samples (100%). Furthermore, the dermal layer in most specimens (95.3%) showed necrosis with heavy infiltration of inflammatory cells mainly neutrophils, lymphocytes and macrophages. The dermal blood vessels of most examined cases (93%) were severely congested with proliferation of endothelial cells together with mononuclear leukocytic cellular infiltration in the blood vessel wall (Fig. 3B). The lesions were extended to subcutaneous tissue together with focal area of hemorrhage were observed in 87% of all examined cases. Moreover, zenker's necrosis of muscular layers which characterized by homogenous and more eosinophilic sarcoplasm with pyknotic nuclei was also noticed in about 50% of the skin samples.

Skin appendages including hair follicles, sebaceous gland and sweat glands of about 75% of skin samples were suffering from degenerative changes manifested by vacuolar and hydropic degeneration with the presence of multiple eosinophilic intracytoplasmic inclusion bodies. Moreover, necrotic

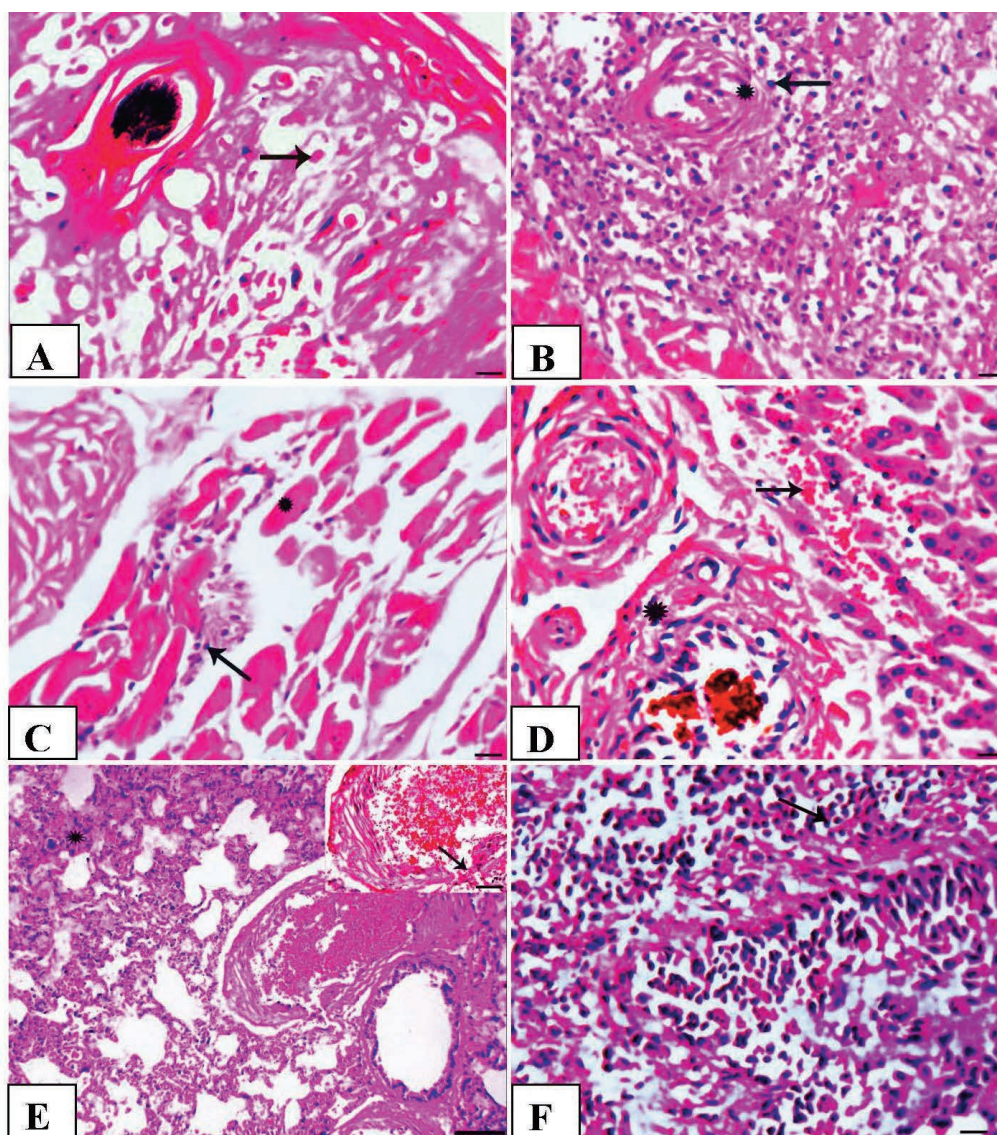


Fig. 3. Representative micrographs for skin (A and B), tongue (C), liver (D), and lung (E and F) from calves naturally infected with lumpy skin disease virus showed: A) Ballooning degeneration of the epithelial cells of epidermis with the presence of intracytoplasmic eosinophilic inclusion bodies (arrow). H&E stain, Bar=20 μ m. B) Vasculitis in an arteriole of the deep dermis with extensive dermal necrosis. H&E stain, Bar=20 μ m. C) Hyaline degeneration of the lingual muscle (asterisk) with few leukocytic infiltrations (arrow). H&E stain, Bar=20 μ m. D) Congestion of hepatic blood vessels with mild periductal fibrous connective tissue proliferation (asterisk) with accumulation of bile pigment in the lumen of bile duct. Note also, hemorrhage in the hepatic parenchyma (arrow). H&E stain, Bar=20 μ m. E) Distention of pulmonary alveoli with eosinophilic fluid (asterisk). Notice also, injury of the intima of pulmonary blood vessels (arrow, inset). H&E stain, Bar=100 μ m, inset Bar=20 μ m. F) Catarrhal bronchitis with peribronchial mononuclear leukocytic cellular infiltration mainly lymphocytes. H&E stain, Bar=20 μ m.

changes of skin appendages were also detected.

Congestion of blood vessels with desquamation of epithelial lining of the tongue papillae was demonstrated in all examined samples. Moreover, hyaline degeneration in the lingual muscle with few leukocytic infiltrations was observed in 66.7% of examined tissues (Fig. 3C). Additionally, focal area of myomalacia was also demonstrated in few samples (50%).

Interestingly, severe thickening and hyalinization of the hepatic capsule as well as congestion of

hepatic blood vessels and blood sinusoids was noticed in 50% of liver samples. Additionally, clear cytoplasmic vacuoles were detected in the cytoplasm of hepatocytes with pyknotic nucleus in the hepatocytes was observed in all examined samples (100%). Desquamation of the epithelial cells lining the bile duct in combination with mild pre-ductal fibrosis with retention of bile pigment in the bile duct was also demonstrated in about 83% of the total examined hepatic tissue (Fig. 3D).

Congestion and thrombosis of the pulmonary

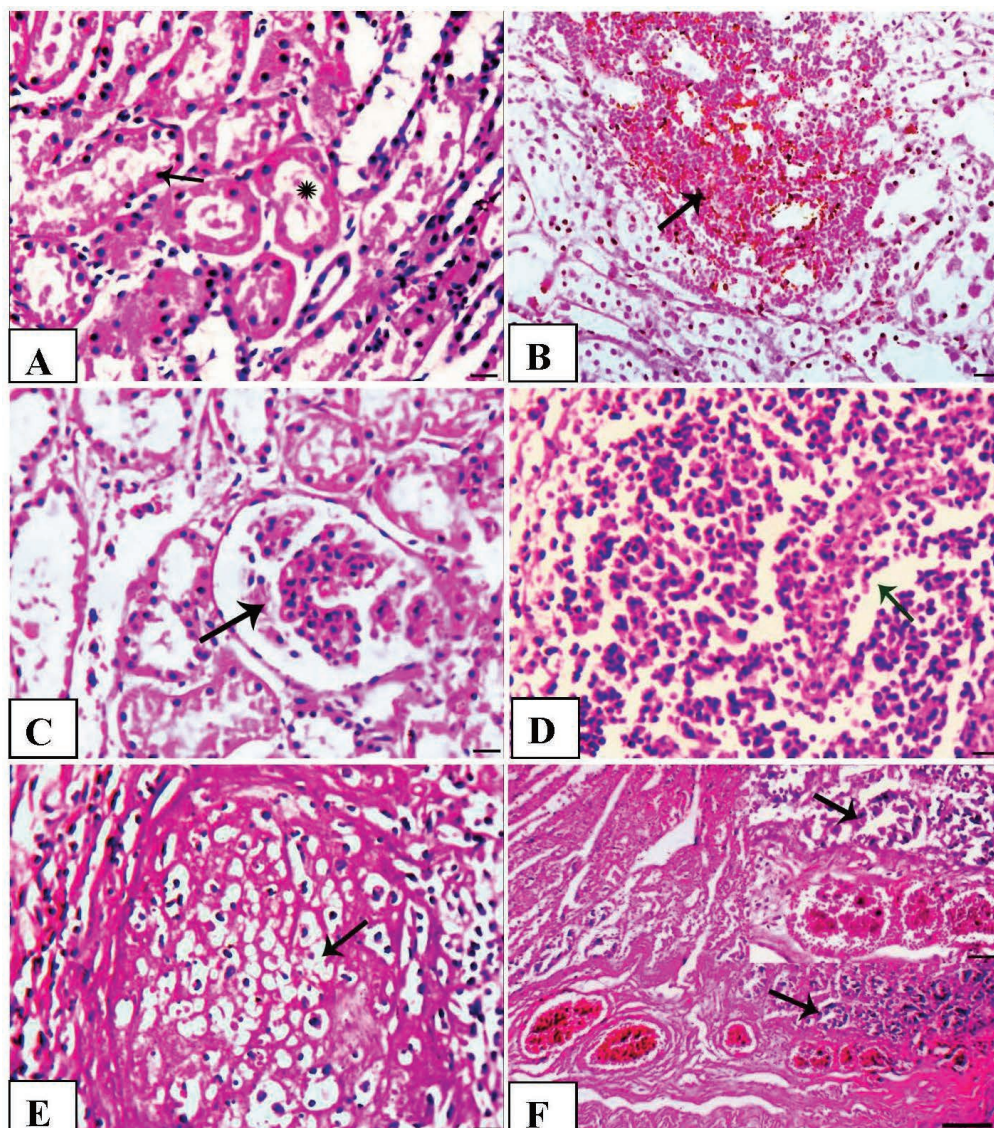


Fig. 4. Representative micrographs for kidney (A, B and C), lymph node (D and E), and intestine (F), from calves naturally infected with lumpy skin disease virus showed: A) Coagulative necrosis of the lining epithelial cells of some renal tubules (arrow) with the presence of eosinophilic debris in the lumen of some renal tubules (asterisk). H&E stain Bar= 20 μ m. B) Focal area of hemorrhage in the renal medulla. H&E stain, Bar=20 μ m. C) Disintegration of the glomerular tuft (arrow). H&E stain, Bar=20 μ m. D) Lymphoid depletion (arrow). H&E stain, Bar=20 μ m. E) Vacuolation of lymphocyte (arrow). H&E stain, Bar=20 μ m. F) Necrosis of the lining epithelium of the intestinal gland with congestion of the submucosal blood vessels (arrow). H&E stain, Bar=100 μ m, inset Bar=20 μ m.

blood vessels as well as thickening of the inter-lobular connective tissue were noticed in 83% of examined pulmonary tissues. Injury in the intima of pulmonary blood vessels with perivascular hemorrhage was also observed. Moreover, accumulation of eosinophilic edematous fluid in the pulmonary alveoli was also observed in 66.7 % of the examined specimens (Fig. 3E). Furthermore, bronchiolitis that characterized by desquamation of the lining epithelium of bronchioles with leukocytic cellular infiltration in the lumen of bronchioles in association with peri-bronchial mononuclear leukocytic cellular infiltration was seen in examined lung samples (100%) (Fig. 3F).

Moreover, congestion of the renal blood vessels with proliferation of the endothelial cell lining of the renal blood vessels was seen in 66.7% of all examined renal tissues. Additionally, necrosis of the epithelial cells lining of the renal tubules with pyknotic nucleus as well as eosinophilic hyaline casts was also detected in the lumen of some renal tubules of all examined kidneys (Fig. 4A). Additionally, few mononuclear leukocytic cellular infiltration mainly lymphocytes and macrophage at corticomedullary junction was demonstrated in some samples (50%). Diffuse areas of hemorrhage the renal tissues with desquamation of the lining epithelium of the renal tubules were noticed in

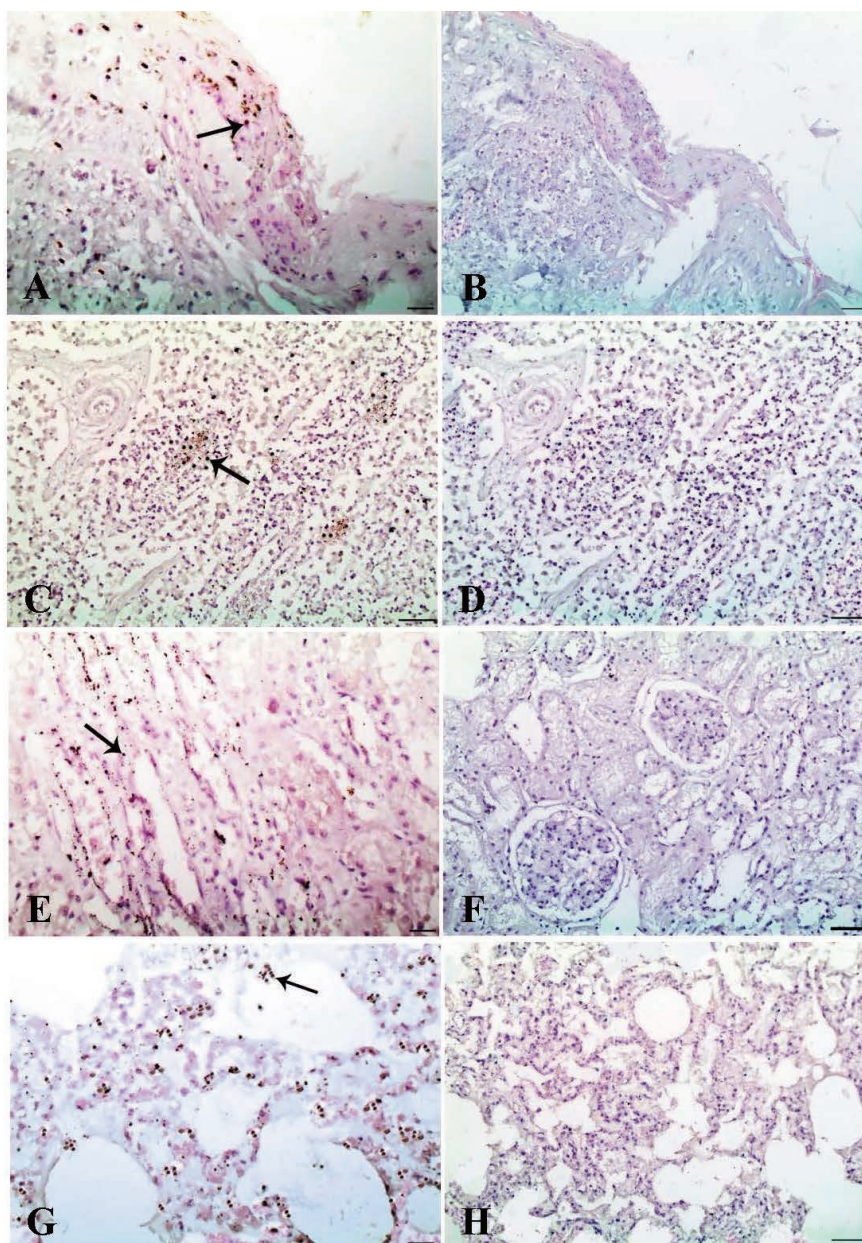


Fig. 5. Positive immunoperoxidase signals for LSDV antigen in (A) The epithelial lining of skin, (B) Skin negative control (C) The lymphoid follicles of lymph node, (D) Lymph node negative control (E) The lining epithelial cells of renal tubules, (F) Kidney negative control (G) The pulmonary alveolar wall, (H) Lung negative control. Bar=20 μ m.

some examined renal samples (66.7%) (Fig. 4B). Furthermore, shrinkage and disintegration of glomerular tuft was also noticed in about 83.3% of all examined kidneys (Fig. 4C).

Severe lymphoid depletion characterized by reduced cellular density of mature small lymphocyte together with necrotic changes in the lymphoid follicles of lymph nodes was demonstrated in all examined lymph nodes (Fig. 4D). Interestingly, polymorphonuclear leukocytic cellular infiltration was demonstrated in the lymphoid follicles. . On the other hand, Degeneration of lymphocytes characterized by vacuolation of lymphocytes with interfollicular edema was also noticed in 66.7% of

the examined samples (Fig. 4E).

Desquamation of the lining epithelial cells of the intestinal villi as well as accumulation of mucoid exudates in the intestinal lumen was seen. Additionally, severe congestion of submucosal blood vessels in association with necrosis of the lining epithelium of intestinal gland was observed in 83.3% of the examined specimens (Fig. 4F).

Immunohistochemistry

LSDV antigen was positively signaled by the presence of brown to dark colour granularity following IHC which may be reflected viral antigen

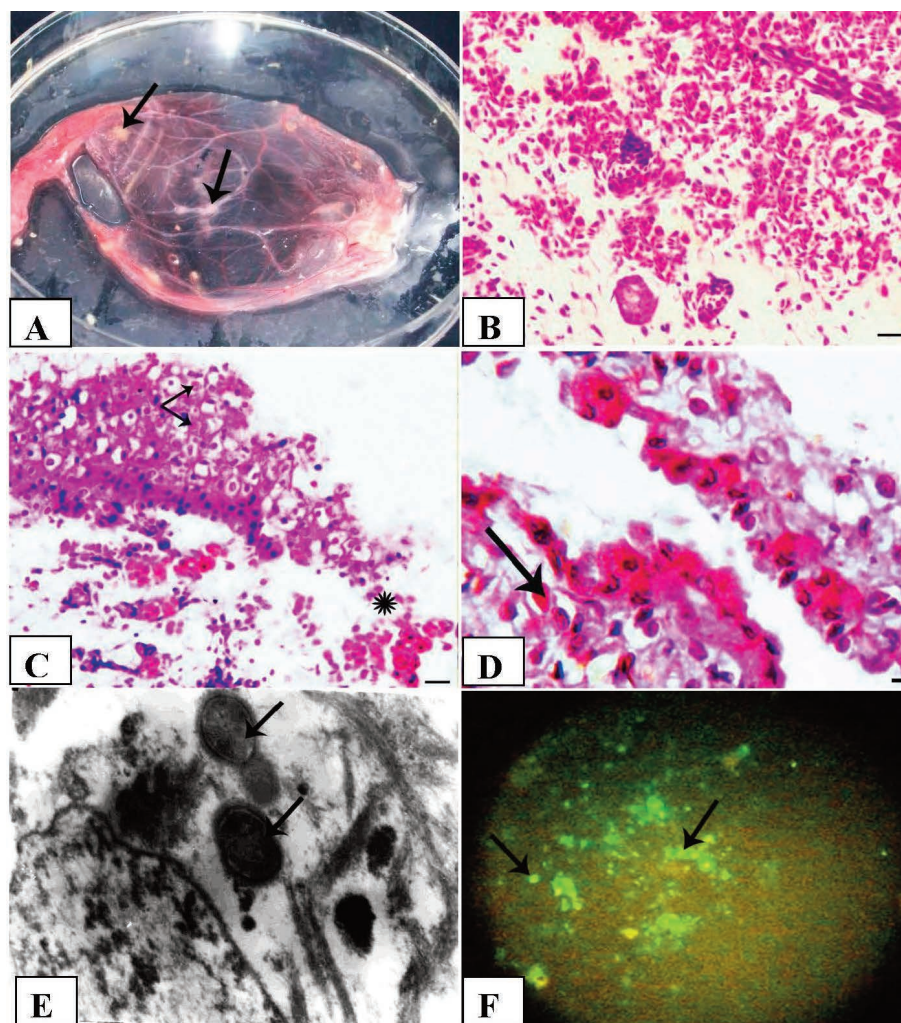


Fig. 6. (A) Macroscopic characteristic pock lesion on CAM of spf ECE. (B-D) Microscopic lesion of LSDV on CAM stained with H&E, (B) Diffuse hemorrhage on CAM (arrow, Bar=20 µm), (C) increase in the thickness of CAM as well as ballooning degeneration of the lining epithelium of CAM with the presence of intracytoplasmic eosinophilic inclusion body (arrow, Bar=20 µm). Note also, destruction of the epithelial layer (asterisk). (D) Intracytoplasmic eosinophilic inclusion body in the lining epithelial cells (arrow, Bar=5 µm). (E) Electron micrograph of LSDV showing the virion appeared ovoid in shape, with rounded ends and characteristic ball of wool appearance, (F) The specific intracytoplasmic yellowish green fluorescent granules on infected CAM stained with IFAT.

or its inclusion bodies in most investigated samples. Following the results of IHC, the skin was the most infected organ as positive reaction was demonstrated in all skin samples (100%). Positive staining was observed in the cytoplasm of the proliferative epithelial cells of skin, hair follicular epithelium (Fig. 5). Additionally, positive immunoperoxidase in lymph nodes (83%), lung (66.7%), and kidneys (50%) was also observed (Fig. 5). Conversely, liver did not show any positive staining in all examined hepatic tissue (6-specimen).

Isolation of LSDV

The results of inoculation of CAMs with the prepared samples from different organs were sum-

marized in Table 1.

Interestingly, the infected CAMs grossly, showed characteristic grayish-white, slightly raised with variable size pock lesion on infected CAMs of spf ECE (Fig. 6A). Additionally, congestion and swelling, accompanied by occasional small hemorrhagic areas were also observed on the infected CAMs. These characteristic pock lesions were noticed after 1st passage and become clear after 3rd passage. Mean while, the examination of these CAMs by light microscope revealed different pathological alterations mainly severe congestion of blood vessels of CAMs. Additionally, diffuse hemorrhage on CAMs (Fig. 6B) was seen. Moreover, multiple variable size pock lesion of LSDV scattered areas on CAMs. Increase in the thickness of CAMs about two or three folds thicker than con-

trol CAMs as well as hyperplasia and hypertrophy with ballooning degeneration of the lining epithelium of CAMs and destruction of the epithelial layer were also seen (Fig. 6C). Interestingly, intracytoplasmic eosinophilic inclusion bodies were detected in hypertrophic and hyperplastic epithelial cells (Fig. 6D). Transmission electron microscopy examination of infected CAMs revealed the presence of virion particles as brick shaped were identified on epithelial cells of the CAMs (Fig. 6E). Moreover, the specific intracytoplasmic yellowish green fluorescent granules characteristic for LSDV was demonstrated by IFAT (Fig. 6F).

Serological identification

Six samples from skin nodules, lymph nodes, lung, liver and kidneys show positive reaction represented in appearance of line of white precipitation with AGPT and also positive results for IFAT with specific antisera for LSDV (Table 1).

Discussion

Lumpy skin disease is generally considered as an economically important disease of cattle in the Middle East and Africa (Fenner, 1996). Although, this disease is of a relatively low mortality rate, but the infection permanently damages the hides, reduced feed intake, mastitis; decreased milk yield; infertility in males and females, decreased semen quality consequently, it is of severe economic importance (Tuppurainen *et al.*, 2005). In the present work, the gross and histopathologic lesions of LSD in skin and different organs of cattle naturally infected with LSDV were applied, aiming to develop the descriptions of lesions reported by others. Furthermore, the presence and localization of LSDV within the affected skin, liver, lung, kidneys and

lymph nodes was evaluated using different molecular methods such as PCR and IHC. Moreover, further isolation of LSDV on spf-ECE with its identification using different techniques was also performed.

LSD is characterized by skin nodules, lymphadenitis with persistent fever. The severity of the disease depends on the dose of the inoculums as well as the susceptibility of the host and the route of exposure as reported by Davies (1981). In the present study, the mortality rate is 6.97% which matched with the findings of Coetzer (2004) who reported that the mortality rarely exceeds 3%, but it can be as high as 40%. Animals suffered from LSD may have less immune response resulting in spreading of the virus in different internal organs.

In the present work, the affected animals showed signs of fever, anorexia, depression, nasal discharge and salivation followed by the sudden appearance of firm skin nodules, all over the body in some cases and enlargement of the superficial lymph nodes were the most common clinical signs. These signs are similar to those described previously in cattle by (Carn and Kitching 1995; Abd El-Rahim *et al.*, 2002). In LSD, the damage of skin or mucous membranes that could be followed by secondary bacterial invasion resulting in various complications involving recumbency, mastitis, abortion, dysentery, lameness, and pneumonia. Additionally, stress induced immunosuppression, anorexia, persistent fever and severe debilitation also facilitate secondary bacterial invasion.

Pathological evaluation of skin lesions at various stages of development is important. Skin nodules have congestion, hemorrhage, edema, and vasculitis with consequent necrosis and involve all layers of the epidermis, dermis, subcutaneous tissue, and often adjacent musculature. The extensive replication of LSDV as indicated by presence of in-

Table 1. results of CAMs inoculation with collected samples from different organs, and serological identification tests (AGPT and IFAT)

Tissue samples	Pock lesion on CAM	AGPT*	IFAT**
Skin nodule	+ve	+ve	+ve
Regionallymph nodes	+ve	+ve	+ve
Lung	-ve	+ve	+ve
Liver	-ve	+ve	+ve
Kidney	-ve	+ve	+ve

*clear precipitation line

**yellowish green fluorescent

tracytoplasmic eosinophilic inclusion bodies in epidermis and germinal cells of affected hair follicles and sebaceous glands. However, vasculitis and thrombosis, resulting in oedema and necrosis was essential to the pathogenesis of the lesions in LSD (Prozesky and Barnard 1982). Lymph nodes draining affected areas were enlarged with mild lymphoid proliferation, edema, congestion and necrosis. These findings are matched with the findings of Prozesky and Barnard (1982).

In the current study, hemorrhage and thrombosis was a common lesion in different investigated organs such as skin, liver, lung, kidney and lymph nodes. These findings could be attributed to the release of cytokines by inflammatory cells (immune-mediated inflammation) or by a direct endothelial damage as observed in the pulmonary blood vessels. LSD lesions can occasionally extend to internal organs (Ali *et al.*, 1990). According to the results of the current work, various pathological changes were detected in different internal organs including lung, liver, kidneys intestine and lymph nodes in which combination with detection of LSDV antigen in most of these organs by IHC. These findings confirm the ability of LSDV to induce systemic infection that did not inadequately confirmed previously. In addition to successful isolation of LSDV from the skin nodule and lymph nodes, further identification of virus by serological tests as AGPT and IFAT were performed. These results are also confirmed the systemic spread of viral antigen in these organs. Moreover, transmission electron microscope examination showed the characteristic ovoid shaped virion with "ball of wool" appearance, the same results were recorded by Ahmed and Kawther (2007).

PCR was a rapid and sensitive mean for detection and identification of the LSDV DNA (Tuppurainen *et al.*, 2005). The use of the PCR for the detection of LSD virus nucleic acid from skin biopsies has been published previously (Markoulatos *et al.*, 2000), but no studies have been undertaken on the presence of the virus in different internal organs. In this study, the PCR assay showed high specificity as a unique band of the expected size (~192 bp) was obtained for DNA samples derived from skin, lymph node, and lung. Following to these results, it is clear that there was a good association between pathological changes in the skin, lymph node and lungs and the identification of viral DNA in these organs by PCR. Meanwhile, LSD

virus could not be demonstrated in liver by PCR, although there were some pathological lesions observed. This may be attributed to the concentration of the virus in the hepatic tissue is too low. Additionally, the demonstration of viral antigen by immunoperoxidase staining in necrotic tissue is significant. Consequently, IHC was applied to demonstrate viral particles in the tissues of infected animals. Overall, IHC was found to be more sensitive than traditional staining methods for demonstration of the causative agent. Moreover, IHC is a method that may be applied to the detection of LSDV in paraffin wax-embedded tissues, as it provides the opportunity to correlate the histological changes with the presence of the virus. However, the uneven distribution of the viral cells within tissues could explain the different results obtained by PCR and IHC, especially in those organs that showed only few viral particles.

Conclusion

Based on the result of the present work, it could be concluded that beside the constant lesion of LSD in skin, systemic spreading of the virus in different organs was reported. PCR and IHC assay should be applied besides conventional techniques for any cases with skin lesions as early as possible to diagnose and apply adequate control measures. Future studies on the cellular tropism of LSDV and the nature of viral receptors on target cell membranes might be useful for more clarification of the pathogenesis of this economic important disease.

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